

SEQUENCE LISTING

<110> Barany, Francis
 Zebala, John
 Nickerson, Deborah
 Kaiser Jr., Robert J.
 Hood, Leroy

<120> A THERMOSTABLE LIGASE MEDIATED DNA AMPLIFICATION SYSTEM
 FOR THE DETECTION OF GENETIC DISEASES

<130> 19603/3641

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<150> US 08/946,458

<151> 1997-10-07

<150> US 08/462,221

<151> 1995-06-05

<150> US 08/343,785

<151> 1994-11-22

<150> US 07/971,095

<151> 1992-11-02

<150> US 07/518,447

<151> 1990-05-03

<150> 09/480,515

<151> 2000-01-10

<160> 47

<170> PatentIn Ver. 2.1

<210> 1

<211> 2111

<212> DNA

<213> Thermus aquaticus ligase

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 aactaccgct actacgtcct ggcggacccg gagatctccg acgccgagta cgaccggctt 180
 cttaggaggac tcaaggagct tgaggagcgc ttccccgagc tcaaaagccc ggactcccc 240

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acccttcagg tgggggagag gcctttggag gccaccttcc gccccgtccg ccaccccacc 300
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atagaacggg ccctggggcg gaaggggccc ttgcctaca ccgtggagca caaggtggac 420
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<210> 2

<211> 676

<212> PRT

<213> *Thermus aquaticus* ligase

<400> 2

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Ile Arg Tyr His Asn Tyr Arg Tyr Tyr Val Leu Ala Asp Pro Glu Ile
    20                      25                     30

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Ser Asp Ala Glu Tyr Asp Arg Leu Leu Arg Glu Leu Lys Glu Leu Glu
    35                      40                     45

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Glu Arg Phe Pro Glu Leu Lys Ser Pro Asp Ser Pro Thr Leu Gln Val
 50 55 60

Gly Ala Arg Pro Leu Glu Ala Thr Phe Arg Pro Val Arg His Pro Thr
 65 70 75 80

Arg Met Tyr Ser Leu Asp Asn Ala Phe Asn Leu Asp Glu Leu Lys Ala
 85 90 95

Phe Glu Glu Arg Ile Glu Arg Ala Leu Gly Arg Lys Gly Pro Phe Ala
 100 105 110

Tyr Thr Val Glu His Lys Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr
 115 120 125

Glu Glu Gly Val Leu Val Tyr Gly Ala Thr Arg Gly Asp Gly Glu Val
 130 135 140

Gly Glu Glu Val Thr Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg
 145 150 155 160

Arg Leu Lys Gly Val Pro Glu Arg Leu Glu Val Arg Gly Glu Val Tyr
 165 170 175

Met Pro Ile Glu Ala Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Arg
 180 185 190

Gly Glu Arg Ile Phe Lys Asn Pro Arg Asn Ala Ala Ala Gly Ser Leu
 195 200 205

Arg Gln Lys Asp Pro Arg Ile Thr Ala Lys Arg Gly Leu Arg Ala Thr
 210 215 220

Phe Tyr Ala Leu Gly Leu Gly Leu Glu Glu Val Glu Arg Glu Gly Val
 225 230 235 240

Ala Thr Gln Phe Ala Leu Leu His Trp Leu Lys Glu Lys Gly Phe Pro
 245 250 255

Val Glu His Gly Tyr Ala Arg Ala Val Gly Ala Glu Gly Val Glu Ala
 260 265 270

Val Tyr Gln Asp Trp Leu Lys Lys Arg Arg Ala Leu Pro Phe Glu Ala
 275 280 285

Asp Gly Val Val Val Lys Leu Asp Glu Leu Ala Leu Trp Arg Glu Leu
 290 295 300

Gly Tyr Thr Ala Arg Ala Pro Arg Phe Ala Ile Ala Tyr Lys Phe Pro
 305 310 315 320

Ala Glu Glu Lys Glu Thr Arg Leu Leu Asp Val Val Phe Gln Val Gly
 325 330 335

Arg Thr Gly Arg Val Thr Pro Val Gly Ile Leu Glu Pro Val Phe Leu
 340 345 350

Glu Gly Ser Glu Val Ser Arg Val Thr Leu His Asn Glu Ser Tyr Ile
 355 360 365

Glu Glu Leu Asp Ile Arg Ile Gly Asp Trp Val Leu Val His Lys Ala
 370 375 380

Gly Gly Val Ile Pro Glu Val Leu Arg Val Leu Lys Glu Arg Arg Thr
 385 390 395 400

Gly Glu Glu Arg Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly
 405 410 415

His Arg Leu Leu Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu
 420 425 430

Cys Pro Ala Lys Arg Phe Glu Ala Ile Arg His Phe Ala Ser Arg Lys
 435 440 445

Ala Met Asp Ile Gln Gly Leu Gly Glu Lys Leu Ile Glu Arg Leu Leu
 450 455 460

Glu Lys Gly Leu Val Lys Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys
 465 470 475 480

Glu Asp Leu Val Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn
 485 490 495

Leu Leu Arg Gln Ile Glu Glu Ser Lys Lys Arg Gly Leu Glu Arg Leu
 500 505 510

Leu Tyr Ala Leu Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn
 515 520 525

Leu Ala Ala Arg Phe Gly Asn Met Asp Arg Leu Leu Glu Ala Ser Leu
 530 535 540

Glu Glu Leu Leu Glu Val Glu Glu Val Gly Glu Leu Thr Ala Arg Ala
 545 550 555 560

Ile Leu Glu Thr Leu Lys Asp Pro Ala Phe Arg Asp Leu Val Arg Arg
565 570 575

Leu Lys Glu Ala Gly Val Glu Met Glu Ala Lys Glu Lys Gly Gly Glu
580 585 590

Ala Leu Lys Gly Leu Thr Phe Val Ile Thr Gly Glu Leu Ser Arg Pro
595 600 605

Arg Glu Glu Val Lys Ala Leu Leu Arg Arg Leu Gly Ala Lys Val Thr
610 615 620

Asp Ser Val Ser Arg Lys Thr Ser Tyr Leu Val Val Gly Glu Asn Pro
625 630 635 640

Gly Ser Lys Leu Glu Lys Ala Arg Ala Leu Gly Val Pro Thr Leu Thr
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Glu Glu Glu Leu Tyr Arg Leu Leu Glu Ala Arg Thr Gly Lys Lys Ala
660 665 670

Glu Glu Leu Val
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<210> 3
<211> 7
<212> PRT
<213> Thermus aquaticus

<400> 3
Asp Ala Glu Tyr Asp Arg Leu
1 5

<210> 4
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Degenerate
Probe

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<222> (3)

<223> Y at position 3 in this sequence can be either c
or t

<220>

<221> unsure

<222> (12)

<223> Y at position 12 in this sequence can be either c
or t

<220>

<221> unsure

<222> (15)

<223> Y at position 15 in this sequence can be either c
or t

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<222> (19)

<223> Y at position 19 in this sequence can be either c
or t

<220>

<221> unsure

<222> (6)

<223> N at position 6 in this sequence can be either a,
g, c, or t

<220>

<221> unsure

<222> (18)

<223> N at position 18 in this sequence can be either a,
g, c, or t

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<221> unsure

<222> (9)

<223> R at position 9 in this sequence can be either g
or a

<220>

<221> unsure

<222> (16)

<223> M at position 16 in this sequence can be either c
or a

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<210> 5
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Oligo Primer

<400> 5
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24

<210> 6
 <211> 130
 <212> DNA
 <213> Artificial Sequence

<220>
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<210> 7
 <211> 2051
 <212> DNA
 <213> Thermus aquaticus ligase

<400> 7
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2051

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<210> 8

<211> 676

<212> PRT

<213> Thermus aquaticus ligase

<400> 8

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Met Thr Leu Glu Glu Ala Arg Lys Arg Val Asn Glu Leu Arg Asp Leu
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Ile Arg Tyr His Asn Tyr Arg Tyr Tyr Val Leu Ala Asp Pro Glu Ile
      20              25              30

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Ser Asp Ala Glu Tyr Asp Arg Leu Leu Arg Glu Leu Lys Glu Leu Glu
      35              40              45

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Glu Arg Phe Pro Glu Leu Lys Ser Pro Asp Ser Pro Thr Leu Gln Val
      50              55              60

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Gly Ala Arg Pro Leu Glu Ala Thr Phe Arg Pro Val Arg His Pro Thr
      65              70              75              80

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Arg Met Tyr Ser Leu Asp Asn Ala Phe Asn Leu Asp Glu Leu Lys Ala
      85              90              95

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Phe Glu Glu Arg Ile Glu Arg Ala Leu Gly Arg Lys Gly Pro Phe Ala
      100             105             110

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Tyr Thr Val Glu His Lys Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr
 115 120 125

Glu Glu Gly Val Leu Val Tyr Gly Ala Thr Arg Gly Asp Gly Glu Val
 130 135 140

Gly Glu Glu Val Thr Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg
 145 150 155 160

Arg Leu Lys Gly Val Pro Glu Arg Leu Glu Val Arg Gly Glu Val Tyr
 165 170 175

Met Pro Ile Glu Ala Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Arg
 180 185 190

Gly Glu Arg Ile Phe Lys Asn Pro Arg Asn Ala Ala Ala Gly Ser Leu
 195 200 205

Arg Gln Lys Asp Pro Arg Ile Thr Ala Lys Arg Gly Leu Arg Ala Thr
 210 215 220

Phe Tyr Ala Leu Gly Leu Gly Leu Glu Glu Val Glu Arg Glu Gly Val
 225 230 235 240

Ala Thr Gln Phe Ala Leu Leu His Trp Leu Lys Glu Lys Gly Phe Pro
 245 250 255

Val Glu His Gly Tyr Ala Arg Ala Val Gly Ala Glu Gly Val Glu Ala
 260 265 270

Val Tyr Gln Asp Trp Leu Lys Lys Arg Arg Ala Leu Pro Phe Glu Ala
 275 280 285

Asp Gly Val Val Val Lys Leu Asp Glu Leu Ala Leu Trp Arg Glu Leu
 290 295 300

Gly Tyr Thr Ala Arg Ala Pro Arg Phe Ala Ile Ala Tyr Lys Phe Pro
 305 310 315 320

Ala Glu Glu Lys Glu Thr Arg Leu Leu Asp Val Val Phe Gln Val Gly
 325 330 335

Arg Thr Gly Arg Val Thr Pro Val Gly Ile Leu Glu Pro Val Phe Leu
 340 345 350

Glu Gly Ser Glu Val Ser Arg Val Thr Leu His Asn Glu Ser Tyr Ile
 355 360 365

Glu Glu Leu Asp Ile Arg Ile Gly Asp Trp Val Leu Val His Lys Ala
 370 375 380

Gly Gly Val Ile Pro Glu Val Leu Arg Val Leu Lys Glu Arg Arg Thr
 385 390 395 400

Gly Glu Glu Arg Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly
 405 410 415

His Arg Leu Leu Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu
 420 425 430

Cys Pro Ala Lys Arg Phe Glu Ala Ile Arg His Phe Ala Ser Arg Lys
 435 440 445

Ala Met Asp Ile Gln Gly Leu Gly Glu Lys Leu Ile Glu Arg Leu Leu
 450 455 460

Glu Lys Gly Leu Val Lys Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys
 465 470 475 480

Glu Asp Leu Val Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn
 485 490 495

Leu Leu Arg Gln Ile Glu Glu Ser Lys Lys Arg Gly Leu Glu Arg Leu
 500 505 510

Leu Tyr Ala Leu Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn
 515 520 525

Leu Ala Ala Arg Phe Gly Asn Met Asp Arg Leu Leu Glu Ala Ser Leu
 530 535 540

Glu Glu Leu Leu Glu Val Glu Glu Val Gly Glu Leu Thr Ala Arg Ala
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Ile Leu Glu Thr Leu Lys Asp Pro Ala Phe Arg Asp Leu Val Arg Arg
 565 570 575

Leu Lys Glu Ala Gly Val Glu Met Glu Ala Lys Glu Lys Gly Gly Glu
 580 585 590

Ala Leu Lys Gly Leu Thr Phe Val Ile Thr Gly Glu Leu Ser Arg Pro
 595 600 605

Arg Glu Glu Val Lys Ala Leu Leu Arg Arg Leu Gly Ala Lys Val Thr
 610 615 620

Asp Ser Val Ser Arg Lys Thr Ser Tyr Leu Val Val Gly Glu Asn Pro
 625 630 635 640

Gly Ser Lys Leu Glu Lys Ala Arg Ala Leu Gly Val Pro Thr Leu Thr
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Glu Glu Glu Leu Tyr Arg Leu Leu Glu Ala Arg Thr Gly Lys Lys Ala
 660 665 670

Glu Glu Leu Val
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<210> 9
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 9
 ctggcttatc gaaattaat 19

<210> 10
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 10
 ccagggtcat tttatTTTct ccatgtacaa at 32

<210> 11
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 11
catggagaaa ataaaatgac cctggaagag gcg 33

<210> 12
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 12
aagccggtcg tactcggc 18

<210> 13
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 13
gtttttcatg gtgcacctga cgcctgg 27

<210> 14
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 14
gtttcatggt gcacctgacg cctct 25

<210> 15
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 15
 gtcattggtgc acctgacgcc tca 23

<210> 16
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 16
 ggagaagtct gccgttactg cc 22

<210> 17
 <211> 51
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: betaA-globin
 forward primer

<400> 17
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<210> 18
 <211> 51
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: betaA-globin
 reverse primer

<400> 18
 ctgtggtacc acgtggactg aggactctc ttacagcggc aatgacggga c 51

<210> 19
 <211> 22
 <212> DNA
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 <220>
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 Oligonucleotide

 <400> 19
 tggtagcacg tggactgagg ac 22

 <210> 20
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

 <400> 20
 tcctcttcag acggcaatga cgtc 24

 <210> 21
 <211> 26
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

 <400> 21
 acctcttcag acggcaatcg cgtttc 26

 <210> 22
 <211> 28
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 22
ccctcttcag acggcaatcg cgtttttc 28

<210> 23
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaA-globin

<400> 23
Met Val His Leu Thr Pro Glu Glu Lys Ser Ala Val Thr Ala Leu
1 5 10 15

<210> 24
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: betaS-globin

<400> 24
Met Val His Leu Thr Pro Val Glu Lys Ser Ala Val Thr Ala Leu
1 5 10 15

<210> 25
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: beta-globin
amplification primer

<400> 25
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<210> 26
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: beta-globin
 amplification primer

<400> 26
 agggcaggag ccagggtgg gg 22

<210> 27
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Alpha1 -
 antitrypsin amplification primer

<400> 27
 tcagccttac aacgtgtctc tgctt 25

<210> 28
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Alpha1 -
 antitrypsin amplification primer

<400> 28
 gtatggctc taaaaacatg gcccc 25

<210> 29
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Cystic
 fibrosis amplification primer

<400> 29
 cagtgaaga atggcattct gtt 23

<210> 30

<211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Cystic
 fibrosis amplification primer

 <400> 30
 ggcatgcttt gatgacgctt ctg 23

 <210> 31
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: betaA-globin
 primer

 <400> 31
 atggtgcacc tgactcctga 20

 <210> 32
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Oligo primer

 <400> 32
 ggagaagtct gccgttactg 20

 <210> 33
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: betaS-globin
 primer

 <400> 33
 atggtgcacc tgactcctgt 20

<210> 34
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Alphal -
 antitrypsin(M) primer

 <400> 34
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 <210> 35
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Oligo primer

 <400> 35
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 <210> 36
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Alphal
 antitrypsin(Z) primer

 <400> 36
 ggctgtgctg accatcgaca 20

 <210> 37
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Cystic
 fibrosis (non-508) primer

<400> 37
attaaagaaa atatcatctt 20

<210> 38
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 38
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<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cystic
fibrosis (508) primer

<400> 39
accattaaag aaaatatcat 20

<210> 40
<211> 23
<212> DNA
<213> Artificial Sequence

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<400> 40
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<210> 41
<211> 25
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<220>
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<210> 42
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<210> 43
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<210> 44
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<210> 45
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<210> 47
<211> 22
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<220>
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Oligonucleotide

<400> 47
caggagtcag gtgcaccatg gt 22